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FEEDING BEHAVIOR IN POLYPS OF THE CHESAPEAKE BAY SEA NETTLE, *CHRYSAORA QUINQUECIRRHA* (DESOR, 1848)

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The medusae of *Chrysaora quinquecirrha* are familiar to fisherman and swimmers in the Chesapeake Bay area, both for their painful stings and for the huge populations extant during the summer months (Cargo and Schultz, 1966). Most of the life cycle of *Chrysaora*, however, is spent as a sessile polyp, 0.5 to 3 mm in length. In their natural environment, the polyps feed on a variety of small invertebrates and fish (Cargo and Schultz, 1966) which they capture with the nematocysts, or stinging organelles, present in their tentacles. Their feeding behavior is similar to that observed in *Hydra* (Lenhoff, 1961) and in a number of other coelenterates (Fulton, 1963; Mariscal and Lenhoff, 1968; Lindstedt, 1971; Reimer, 1971; Williams, 1972) and can generally be described as follows. Upon stimulation the tentacles contract and bend towards the mouth. The mouth then opens and, in most cases, the tentacles pass through the mouth opening and enter the gut cavity. The latter situation is described in this paper as tentacle stuffing and is shown in Figure 1.

It has long been known that food extracts can elicit such behavior in coelenterates (Nagel, 1892). In 1955, Loomis demonstrated that the tripeptide, reduced glutathione, specifically stimulated the feeding response in *Hydra*. Since that time the amino acids proline (Lenhoff, 1968; Reimer, 1971), valine (Lindstedt, Muscatine and Lenhoff, 1968), asparagine (Lindstedt, 1971), glutamine (Lenhoff, 1968; Williams, 1972), alanine (Reimer, 1971), serine (Williams, 1972), aspartic acid (Williams, 1972), histidine (Williams, 1972), tryptophan (Williams, 1972), and lysine (Reimer, 1971), acting either alone or in combination with reduced glutathione (Lenhoff, 1968; Lindstedt, 1971; Reimer, 1971), have been cited as specific activators of the feeding response in several other coelenterates. In addition, tyrosine, when present in the gut of *Hydra*, causes a modification of the normal feeding response (Blanquet and Lenhoff, 1968).

Information about the chemical nature of specific feeding response activators in coelenterates has generally concerned the classes Hydrozoa and Anthozoa (Lenhoff, 1968) although Muscatine (Lenhoff, H. M., University of California at Irvine, personal communication) has found that reduced glutathione induces feeding behavior in polyps of the scyphozoan, *Aurelia*. The data presented here for the polyp stage of *Chrysaora quinquecirrha* is the first comprehensive study of artificially induced feeding behavior in the class scyphozoa. It is reported in this paper that most naturally occurring amino acids and several small peptides elicit feeding movements in *Chrysaora* polyps. Studies were undertaken to determine

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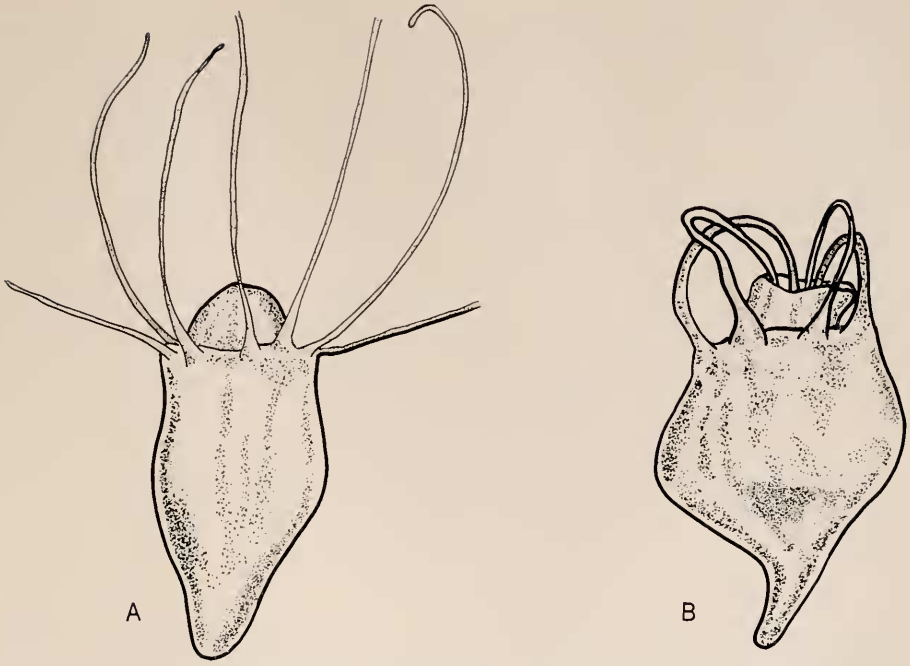

FIGURE. 1. Feeding behavior in *Chrysaora* polyps; (A.) polyp with closed mouth and outstretched tentacles prior to introduction of feeding stimulant; (B.) polyp after exposure to 10^{-5} Molar reduced glutathione. Most of the tentacles have been omitted from the drawings for clarity.

the characteristics of *Chrysaora's* feeding receptor system and the active sites on the amino acids and peptide molecules which stimulate the receptors to activate the pattern of feeding behavior.

MATERIALS AND METHODS

Laboratory cultures of polyps of *Chrysaora quinquecirrha* known as Type 2 (Loeb, 1972) were used in these studies. Artificial seawater (Instant Ocean, Aquarium Systems Inc. Wickliffe, Ohio), prepared as a solution of 13 parts per thousand salt, served as the culture medium. All substances tested were dissolved in and subsequently diluted with this medium; they were initially prepared as 10^{-4} M solutions to approximate the concentration of amino acids in crustacean hemolymph (Florey, 1966; Srinivasagam, Raymont, Moodie and Raymont, 1971). Amino acids, sugars, and reduced glutathione were obtained from Sigma Biochemicals, St. Louis, Mo. Substituted amino acid analogues were from Nutritional Biochemicals, Cleveland, Ohio; ethanolamine and ethylene diamine were from Matheson, Coleman and Bell Inc., Norwood, Ohio; urea from Schwartz-Mann Research Laboratories, Orangeburg, New York, acetaldehyde and valeraldehyde from Eastman Kodak, Rochester, New York. Trypsin and bacitracin were from Calbiochem, Los Angeles, California.

Brine shrimp extract was prepared by homogenizing 100 recently hatched (24 to 48 hours old) shrimp nauplii in a few drops of artifical seawater with the aid of a hand held glass microhomogenizer. Following dilution to 1 ml with artificial seawater, the homogenate was centrifuged 15 minutes at 5000 rpm; the clear supernatant was serially diluted with artificial seawater for use in subsequent experiments. In order to correlate the amino acid concentration of brine shrimp homogenate to the amino acid and glutathione solutions tested, several assumptions were made concerning the homogenate. Several sources (Florey, 1966; Srinivasagam *et al*, 1971) report the amino acid content of the body fluids of small crustacea to be about 10^{-4} M. The weight of the average 24 hour brine shrimp nauplius was determined to be 7.2 ± 0.8 s.d. micrograms. This was accomplished by weighing six individual lots of ten well drained brine shrimp nauplii on a Cahn electrobalance, and then calculating the mean weight of one nauplius. Assuming that the density of each brine shrimp was one and that each brine shrimp was constructed solely as a container holding 10^{-4} M amino acid solution, the concentration of any dilution of brine shrimp homogenate could be roughly estimated. The protein content of the supernatant was determined by the method of Lowry, Rosebrough, Farr and Randall (1951), using bovine serum albumin as the stanard protein.

For each test five *Chrysaoroa* polyps, starved for one week prior to use, were removed from a stock culture and placed in approximately 5 ml of test solution in a small stender dish. Observations were begun immediately with the aid of a dissecting microscope. Each test was repeated one or more times. All work was done at room temperature (23–25° C). Since most of the time taken by feeding behavior involved insertion of tentacles into the mouth and thence into the body cavity (stuffing), feeding time was defined as the time from initial tentacle insertion to complete tentacle withdrawal. Other effects such as tentacle writhing or unusually wide mouth opening (gaping) were recorded as observations. Complete tentacle withdrawal was, in most cases, accompanied by mouth closing and was therefore considered the end of the response. On several testing occasions particularly sensitive polyps exhibited slight feeding responses when dropped into fresh artificial seawater; this time was therefore subtracted from feeding times observed under experimental conditions, and does not appear in the data.

In order to obtain an insight to the location of receptors involved in the feeding behavior patterns, tentacles were cut from two or more animals within 30 seconds after their immersion in the test solution and observed in the same dish as the whole polyps. Tentacles removed in seawater and then placed in minimally effective concentrations of glutathione, tyrosine, phenylalanine, glutamine and cystein gave the same responses as tentacles cut and observed in these solutions.

Results

General feeding responses were noted for all amino acids tested except lysine. It should be noted that the intensity and duration of the whole response varied with the substance tested; in addition, the intensity of individual components of the response varied. Thus, in some instances, the tentacles would writhe vigorously prior to bending towards the mouth. The mouth might open

slightly or gape widely. The extent and duration of tentacle stuffing was dependent on the stimulating substance. The minimal effective concentration for amino acids or small peptides ranged from 10^{-4} M to 10^{-12} M, depending on the chemical being tested. These results are presented in Table I. A commercial protein hydrolysate (Bacto-Peptone, Difco Laboratories, Detroit, Mich.), also induced feeding behavior at a minimum concentration of 0.02 mg/ml (approximately 2×10^{-4} M amino acids if all of the hydrolysate is assumed to be amino acids). No feeding response was observed in the presence of large peptides such as bacitracin (m.w. 1400) (Sober, 1968) or to proteins such as bovine serum albumin or trypsin.

Isolated tentacle responses reflected whole animal responses; tentacle writhing or contraction or elongation occurred in most amino acid and peptide solutions, as noted in Table I. In contrast, control tentacles excised in fresh medium showed little or no contraction and, propelled by ciliated cells (Chuin, 1930), slowly moved about the dish. Tentacles exposed to a noxious substance such as weak hydrocloric acid merely contracted.

Chrysaora polyps also exhibit the feeding response when presented with a cell-free extract of brine shimp nauplii. However, the characteristics of the response are dependent on the concentration of the extract in the test solution. At

TABLE I

Effect of amino acids and peptides on feeding behavior. The symbols may be interpreted as follows: W, tentacle writhing; S, tentacle stuffing; E, tentacle elongation; C, tentacle contraction; G, gaping mouth; O, no effect; (—), test not performed

	Effect (whole animal)	Effect (excised tentacle)	Minimum effective concentration	Effective time at lowest concentration (minutes)
Alanine	W, G, S	C	10^{-8}	30
Arginine	S	O	10^{-4}	15
Asparagine	G, S	—	10^{-4}	15
Aspartic acid	G, S	—	10^{-4}	10
Cystein	G, S	O	10^{-8}	25
Glutamine	W, G, S	W	10^{-8}	30
Glutamic acid	W, G, S	E	10^{-8}	60
Glycine	S	—	10^{-6}	15
Histidine	G, S	E	10^{-8}	35
Isoleucine	G, S	—	10^{-4}	60
Leucine	S	—	10^{-4}	15
Lysine	O	—	—	—
Methionine	S	W	10^{-6}	35
Phenylalanine	G, S	W	10^{-4}	40
Proline	W, G, S	W	10^{-8}	20
Serine	S	—	10^{-4}	15
Threonine	S	W	10^{-8}	25
Tryptophan	G, S	E, W	10^{-8}	55
Tyrosine	G, S	E	10^{-4}	15
Valine	W. S	W	10^{-8}	50
Glutathione (GSH)	W, G, S	See text	10^{-12}	15
Glycylglycine	G, S	E	10^{-8}	35

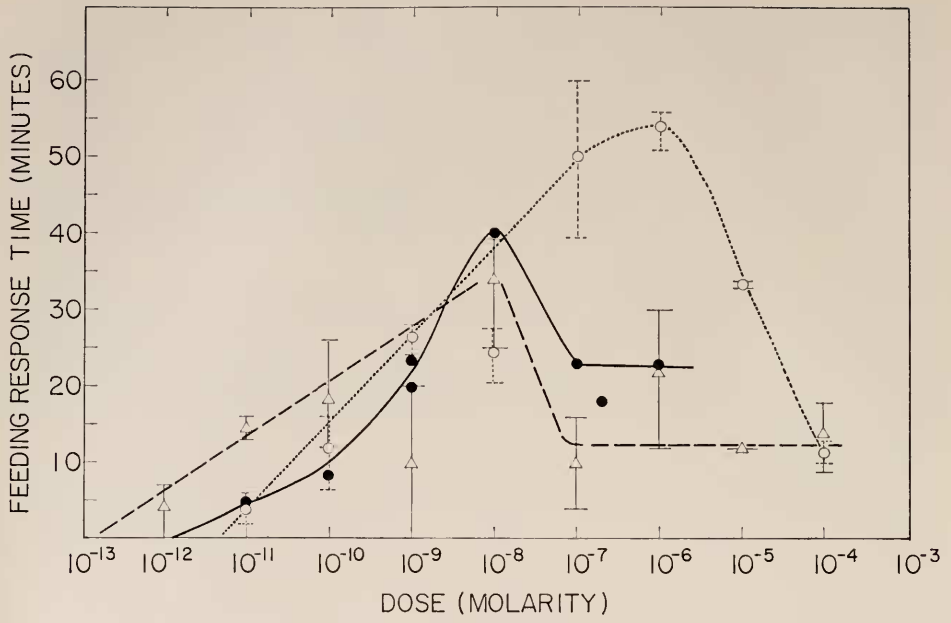

FIGURE 2. Differences in feeding response time as the concentration of feeding behavior stimulus is changed. Open symbols equal response to reduced glutathione. Bars represent the spread in data; each symbol represents the mean feeding time for 10 polyps. Closed symbols equal response to brine shrimp homogenate supernatant. Each symbol represents the mean feeding time for 10 polyps. Data spread omitted for clarity. The dose represents the approximate amino acid concentration of shrimp homogenate and the specific concentrations of the glutathione solutions.

high concentrations (about 10^{-6} M amino acids or 13γ of protein per ml of test solution) tentacle writhing, mouth opening, and tentacle stuffiing occurred simultaneously. At somewhat lower concentrations (approximately 10^{-7} to 10^{-9} M amino acids) the onset of tentacle writhing was delayed for a few seconds after the polyps were introduced to the test solution. Mouth opening and tentacle stuffing took place from one to three minutes later. At even lower concentrations of extract (approximately 10^{-9} to 10^{-12} M amino acids), writhing began one to three minutes after exposure to the test solution, mouth opening one to 10 minutes after exposure, and tentacle stuffing 12 to 24 minutes after exposure. In contrast, reduced glutathione elicited simultaneous tentacle writhing, mouth opening and tentacle insertion at all active concentrations.

As shown in Figure 2, the feeding response time increases as the concentration of extract increases, from a concentration of approximately 10^{-12} M amino acids to approximately 10^{-8} M amino acids, and then falls off to lower response times as the concentration of extract increases further. Similar curves were generated by testing known concentrations of reduced glutathione, and are also shown in Figure 2. All polyps were starved for one week prior to testing; each point on the curve represents the mean feeding response time of 10 polyps.

It is interesting to note that isolated tentacles placed into 10^{-4} and 10^{-5} M reduced glutathione are inactive. However, writhing does occur in glutathione solutions of 10^{-6} to 10^{-10} M, indicating that the response is inhibited at higher concentrations of reduced glutathione.

All common amino acids, with the exception of lysine, induce feeding behavior. Proline, glutamic acid and alanine prepared with acetylated α amino groups, and proline, alanine and tyrosine prepared with esterified α carboxyl groups, as well as the substituted amino acid thioproline, all elicited feeding behavior with feeding times comparable to their natural counterparts. The sugars glucose and glucosamine at 10^{-4} M produced no feeding behavior. The amino alcohol, ethanolamine, or the diamine, ethylenediamine, at 10^{-2} to 10^{-4} M, produced no response. Aldehydes such as acetone, acetaldehyde, and valeraldehyde elicited no feeding responses. However, 10^{-3} M urea produced a mean stuffing response time of 42 minutes; 10^{-4} M urea elicited stuffing for a mean time of 30 minutes. However, acetone produced no effect. Some organic acids also induced feeding; α ketoglutarate, 10^{-4} M at pH 7 elicited feeding for 15 minutes; pyruvate and lactate at 10^{-5} M elicited feeding for 30 minutes. Succinate induced no feeding behavior at 10^{-4} M.

Discussion

Chrysaora polyps initiate feeding behavior in response to most naturally occurring amino acids and to some small peptides. This is surprising, since most other coelenterates previously studied respond to only one or two specific compounds (Lenhoff, 1961, 1968; Mariscal and Lenhoff, 1968; Reimer, 1971; Lindstedt, 1971). However, Forrest (1962) suggests multiple feeding activators in several species of *Hydra*, and Goreau, Goreau and Yonge (1971) present evidence that at least four amino acids (glycine, alanine, phenylalanine and leucine) in concentrations as low as 10^{-9} M induce feeding behavior in several species of reef coral. Williams (1972) also showed that some expression of feeding behavior occurs in the sea anemone *Diadumene* in response to six amino acids, reduced glutathione, pyridoxine, and nicotinic acid. However, the concentrations of these chemicals needed to evoke feeding behavior were, with the exception of aspartic and glutamic acids, in the order of 10^{-3} to 10^{-1} Molar. *Chrysaora* responded to activating substances at concentrations of 10^{-4} M or less, responding to reduced glutathione at 10^{-12} M. The concentration of free amino acids in the body fluids of small crustacea, common prey of *Chrysaora* polyps, is about 10^{-4} M (Florey, 1968; Srinivasagam *et al.* (1971). Thus the amount of material needed to initiate feeding behavior in *Chrysaora* polyps corresponds more closely to the amounts of amino acid one would expect to find in the seawater when nematocysts puncture the exoskeltons of crustacean prey.

Chrysaora polyps respond to increasing amounts of either brine shrimp extract or reduced glutathione with a linear increase in feeding time to a maximum value, followed by an inhibition of the response as the concentration of extract or glutathione continutes to rise, as shown in Figure 2. The differences in the concentration of glutathione necessary to cause inhibition of the feeding response cannot be explained at this time, but may be due to inherent variability between the groups

of polyps used in the tests, even though all polyps were starved for one week prior to testing. However, the same general shape of all of the curves shown in Figure 2 indicates that inhibition of the feeding response occurs at higher concentrations of brine shrimp extract and reduced glutathione. In contrast, Lenhoff (1961) showed that *Hydra* respond to increasing amounts of glutathione by increasing the time spent in feeding behavior up to a steady state maximum value, indicating that stimulation of all the available receptors occurs in the presence of an excess of reagent. It appears that maximum stimulation of feeding receptors in *Chrysaora* results in less than maximum response.

In many cases, isolated tentacles exposed to amino acids and peptides reacted in a manner corresponding to tentacle behavior in the intact polyps, either by writhing or extension or both. The data suggests that at least some feeding reflex receptors are present on the tentacles and that simple reflex behavior can result from receptor stimulation. The hypothesis is further supported by data showing that glutathione at concentrations 10^{-6} M and higher was inhibitory; tentacle writhing only occured at concentrations less than 10^{-6} M reduced glutathione. This tentacle behavior is similar to that observed in whole animals. The presence of receptors on the tentacles would seem to be an evolutionary advantage, as tentacle cells are the first to contact substances emanating from prey animals punctured by tentacle nematocysts.

The minimal effective concentration for each amino acid tested varied and no correlation could be made between this concentration and the size or composition of the side groups of these amino acids. Peptides made up of two (glycylglycine) and three (reduced glutathione) amino acids induced feeding behavior. However, a peptide of 12 residues (bacitracin) and proteins such as serum albumin and trypsin did not induce feeding. Therefore it appears that a peptide which can successfully stimulate feeding behavior receptors must be of low molecular weight.

The inability of amines, aldehydes or alcohols to stimulate feeding indicates that these chemical groups alone are not responsible for activation of feeding behavior. However, the response to urea but not to acetone indicates that an amino-keto combination will initiate feeding behavior. Acetylation of the amino nitrogen groups and esterification of carboxyl groups of a number of amino demonstrates that free amino and carboxyl groups are not necessary to elicit the feeding response. Because *Chrysaora* respond to several types of amino acid and peptide-like compounds, it is possible that a number of receptor sites sensitive to a spectrum of amino acids and small peptides exist which elicit the same general pattern of feeding behavior. Minor differences in response to individual amino acids, such as control over the extent of mouth opening or tentacle writhing, may reflect this situation. Further evidence comes from the observation of the sequence of events exhibited by *Chysaora* polyps in response to the mixture of nutrients available in a dilute brine shrimp homogenate supernatant; in the lowest active concentration tentacle writhing occurred first, followed somewhat later by mouth opening, and still later by insertion of tentacles into the gut cavity. In the presence of a single agent, glutathione, all events occurred simultaneously, even at the lowest stimulatory concentration. Therefore, it is suggested that the orderly activation of each of the

events in this behavioral sequence depends on the activation of receptors by more than one of the naturally occurring amino acids and peptides, even though each substance alone can activate the entire sequence if it is present in sufficient quantity, as shown in Table I. This hypothesis is supported by the work of Reimer (1971). She showed that the sea anemone, *Palythoa,* will exhibit feeding behavior when presented with filter paper soaked in relatively high concentrations of either proline or reduced glutathione (10^{-2} to 10^{-3} M). However, solutions containing both glutathione and proline were either mutually inhibitory or synergistic, depending on the proportion of each substance present. Induction of the correct food catching, mouth opening, and food swallowing sequence occurred with a particular proline-glutathione combination which was effective at a concentration approximately two orders of magnitude less than either component alone. The data suggests that different receptors in *Palythoa* respond to high concentrations of either proline or reduced glutathione indiscriminately, but respond to low concentrations of one or the other substance in a more specific manner. The end result is a modulation of the feeding response. In contrast, a clear separation of feeding behavioral events in response to chemical stimuli is shown by Lindstedt (1971); the sea anemone *Anthopleura* responds to the amino acid, asparagine, by contracting and bending its tentacles toward its mouth but requires stimulation by reduced glutathione in order to ingest the food once it arrives at the mouth. Regulation of feeding behavior in *Chrysaora* may be analagous to that described by Reimer (1971) for *Palythoa.*

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Summary

1. Polyps of *Chrysaora quinquecirrha* exhibit characteristic feeding behavior in response to low concentrations of most common amino acids and to several small peptides.

2. Isolated tentacles also respond in characteristic fashion to amino acids and peptides. The data imply the presence of feeding reflex receptors on the tentacles.

3. Increasing concentrations of brine shrimp extract or reduced glutathione induce longer feeding response times until a maximum value is reached; further increases in extract or reduced glutathione concentration are inhibitory to the response. Thus maximum stimulation of feeding reflex receptors is inhibitory to the feeding behavior response.

4. It was not possible at this time to characterize a specific active site in amino acids or peptides which induces feeding behavior in *Chrysaora* polyps. The data suggest that *Chrysaora* possesses more than one type of receptor, and thus can interact with a number of amino acids and peptides to bring about orderly, modulated, feeding behavior.

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